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14. ABSTRACT
A recent hypothesis suggests a link between chronic inflammation within the prostate and the development and progression of prostate cancer (PCa). Inflammation in the prostate is common and takes many forms including prostatitis, proliferative inflammatory atrophy (PIA), and elevated markers of inflammation, such as interleukin-6. Prior literature suggests that cancer risk increases in the presence of chronic injury to tissues, as can result from chronic inflammation. Indeed, prostate cells affected by inflammation, e.g., PIA, show changes in their DNA similar to those seen in prostatitic intraepithelial neoplasia, a potential pre-cancerous lesion, and in PCa itself. This study aims to test the hypothesis that SNP alleles of selected inflammation-related genes increase risk of PCa. A second aim is to determine the role of SNPs in these genes on risk of developing more aggressive forms of PCa. The third aim is to explore the role of these alleles on risk of dying of PCa. At present, men from two population-based case-control studies in King County, Washington have been genotyped for 146 SNPs in fourteen candidate genes. Cases were diagnosed between January 1, 1993 and December 31, 1996 and between January 1, 2002 and December 31, 2005, respectively. Genotyping has been completed for 1,457 cases and 1,351 controls, with 141 of 146 SNPs successfully genotyped with ≥ 95% completeness and 99% agreement between blind duplicates. Hardy-Weinberg equilibrium has been calculated for all SNPs and linkage disequilibrium statistics (D' and r2) have been calculated for all SNPs within the same gene. Preliminary analyses are under way and odds ratios, adjusted for age alone, have been calculated for each SNP as an estimate of the association between the SNP genotypes and risk of prostate cancer.

15. SUBJECT TERMS
prostate cancer, epidemiology, association study, inflammation

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Introduction

Prostate cancer imposes a large public health burden on society, with more than 200,000 men diagnosed each year. Despite this, there are few established risk factors. Importantly, not all men diagnosed with prostate cancer develop clinically aggressive disease, but there is currently no way to distinguish these men from men who will progress to important disease. A recent hypothesis suggests a link between chronic inflammation within the prostate and the development and progression of prostate cancer. A large body of evidence suggests that cancer risk increases in the presence of chronic injury to tissues, such as can be caused by chronic inflammation and infections leading to inflammation. In the prostate inflammation is common and takes many forms including prostatitis, proliferative inflammatory atrophy (PIA), and elevated markers of inflammation, such as interleukin-6. Cells affected by inflammation, e.g., PIA, show changes in their DNA that appear similar to those seen in prostatic intraepithelial neoplasia, a potential pre-cancerous lesion, and in prostate cancer itself. The results of studies in high-risk prostate cancer families also support the existence of a link between inflammation and this cancer, as two of the candidate genes identified, i.e., RNaseL and MSR1, play roles in inflammation-related processes. The main goal of this study is to test the hypothesis that alleles of selected genes in inflammation-related pathways increase the probability of developing prostate cancer. The specific aims proposed are:

(1) To determine the role of SNPs and haplotypes in select genes involved in inflammation-related processes (i.e., AKT1, CXCR4, CXCL12, IL4, IL6, IL6R, IL6ST, IL8, IL10, NFKB1, PIK3R1, PTGS2, STAT3, TNF, VEGF) on prostate cancer risk. This includes investigating whether the risk of prostate cancer associated with these alleles varies according to race, family history of prostate cancer, or age at diagnosis, or the presence of a second allele (from the above genes).

(2) To determine the role of SNPs and haplotypes in selected genes involved in inflammation-related processes on risk of developing more aggressive forms of prostate cancer (i.e., high Gleason score, advanced stage, or pre-treatment PSA >20 ng/mL).

(3) To explore the role of the aforementioned genes on risk of dying of prostate cancer.

Participants from two population-based case-control studies in King County, Washington were genotyped. Cases were diagnosed with histologically confirmed invasive PCa between January 1, 1993 and December 31, 1996 and January 1, 2002 and December 31, 2005, respectively, and controls were selected from the same underlying population using random digit dialing. Genotyping has been completed for 1,457 cases and 1,351 controls, representing 82% and 83%, respectively, of all men interviewed. Analyses of this data and of demographic and lifestyle data collected by in-person interviews is currently underway. The work and progress described here provide an essential component of the predoctoral training of the grant awardee. The successful completion of the doctoral degree and research goals listed will provide me with sufficient experience to begin a career path of independent research as a cancer epidemiologist in the field of prostate cancer research.

Body

As described in the specific aims above and in the original grant application, the core of the training program will consist of data generation and analyses. In the initial Statement of Work, data analysis was based on 564 controls and 631 cases with DNA available. These men were participants in a population-based case-control study of prostate cancer, with cases diagnosed between January 1, 1993 and December 31, 1996 (1). DNA from a second population-based case-control study, conducted in the sample geographic area as the first study, became available for the analysis. Thus, an additional 787 controls and 827 cases, diagnosed between January 1, 2002 and December 31, 2005, were successfully added to the genotyping effort described previously. As a result, genotype data is available on 1,457 cases and 1,351 controls. This key addition to the originally proposed study will greatly enhance the opportunity for success of this analysis by increasing the statistical power available to detect a significant association between SNP alleles and prostate cancer risk. No additional costs will be incurred as a result of this addition.

Six tasks were described in the approved grant proposal Statement of Work, with three tasks to be undertaken in the first year of the training grant. Task 1, the selection of tagSNPs to capture genetic variation in 14 selected inflammation-related genes (see Appendix I Inflammation-related Genes and SNPs) and subsequent genotyping, has been successfully completed. Task 2, preparation of the data for analysis and preliminary evaluation including assessment of quality control measures has also been successfully completed. Of the 146 SNPs attempted, three SNPs failed on the ABI SNPlex™ genotyping platform and two proved monomorphic in the study sample population. Evaluation of quality control measures showed 99% agreement across 145 blind duplicates, with >95% completion across all SNPs. Task 3, evaluation of prostate cancer risk associated with SNP alleles in inflammation-related genes is

currently underway. In the course of preparing statistical programs to analyze the SNP data for this study, an additional analysis was carried out on an unrelated, pilot set of SNPs (see below and Reportable Outcomes). This was a very useful exercise that allowed me to gain greater proficiency with SAS programming and create a program to analyze large numbers of SNPs rapidly and efficiently. The results of this pilot analysis have been submitted for publication and the manuscript is awaiting results of the review process.

For Task 3, in order to address the specific requirements of evaluating risk associated with haplotypes, I familiarized myself with computer programs that estimate haplotypes and their associated risks, i.e., haplo.stat (2), PHASE (3), and Hplus (4). As each program uses different algorithms to estimate haplotypes, comparing results between programs has been useful for validation and to further my understanding of haplotype estimation methods. I have also had opportunity to consider the merits of different methods to incorporate multiple SNPs from the same gene, i.e., the use of haplotypes versus including separate covariates for each SNP within a regression model. A future challenge will be to investigate statistical methods that allow SNPs from different genes to be considered in the same model, i.e., methods to evaluate gene-gene interactions. This analysis is directly in line with my research interests, which are to understand the role that genetics plays in the etiology of complex disease, such as prostate cancer, and contributes directly to expanding my experience with current methodologies in genetic association studies.

The scientific environment of the Fred Hutchinson Cancer Research Center has made a wide range of interdisciplinary expertise available to me. The opportunity to consult with individuals with expertise in genetic epidemiology and association studies as well as with statistical methods has been especially useful during the analysis portion of the training grant, but all collaborators of this training project have been highly supportive. Dr. Elaine Ostrander, in particular, has provided tremendous support with the genotyping for this proposal, by including genotyping additional cases and controls from a second population-based study. The strong support of my mentor, Dr. Janet Stanford, has also been invaluable. Her keen interest in all the subject areas of this training grant and epidemiological expertise provide me with ongoing guidance and inspiration. In addition, during this first year of the training grant, I have completed a certificate in Research Ethics offered by the FHCRC. I also continue to participate in journal clubs and seminar series at the FHCRC and University of Washington, including the monthly Program in Prostate Cancer Research seminars (see Appendix II for attached PPCR 2008 seminar schedule) and the monthly prostate program discussion meetings. The seminar series invites leading experts in prostate cancer research to speak and allows for an unparalleled opportunity to interact with key players in this field. In the upcoming year, I plan to participate in the newly developed FHCRC Genetic and Molecular Epidemiology Group discussions and will attend a new University of Washington course in genetic association studies.

Key Research and Training Accomplishments to Date

Task 1: Status – Completed.

- Identification and selection of tagSNPs for genotyping in inflammation-related genes
- Design of SNPlex arrays for genotyping of selected tagSNPs
- Expansion of dataset to include additional samples (787 controls, 827 cases)
- Successful genotyping of 141 SNPs in inflammation-related genes,
  - 141 tagSNPs successfully genotyped
  - 99% agreement between 145 blind duplicate samples

Task 2: Status – Completed.

- Preparation of data for analysis: data cleaned and prepared for analysis
- Evaluation of Hardy-Weinberg equilibrium and and calculation of linkage disequilibrium for SNPs within the same gene, using SAS Genetics model 9.1.3 and Haploview programs
- Creation of SAS program templates :
  1. to manipulate data formats for import into additional computer programs
  2. to allow efficient calculation of genotype distributions across case and controls and calculation of odds ratios from logistic regression analysis

Task 3: Status – Underway.

- Familiarization with several common computer programs designed to allow haplotype estimation from unphased genotype data and subsequent calculation of associated odds ratios for those haplotypes, i.e., fastPHASE, Hplus 2.5, and haplo.stats.
- Estimation of initial odds ratios adjusted only for age, a component of the study design, for all SNPs

Tasks 4-6: Status – Projected for completion in Year 2 of the training grant.

Additional Items: Status – Completed.

- An analysis of unrelated SNPs was carried out as a pilot test of the analysis programs and approach prior to the completion and availability of genotyping for the inflammation-pathway related SNPs. This effort has been described in a manuscript and submitted for publication (see Reportable Outcomes below).
- Completion of the Research Ethics Education Program Certificate offered by the FHCRC to trainees.

Reportable Outcomes

- **Manuscript**: Multiple Independent Genetic Variants in the 8q24 Region are Associated with Prostate Cancer Risk. Claudia A. Salinas, Erika Kwon, Chris Carlson, Joe Koopmeiners, Ziding Feng, Danielle M. Karyadi, Elaine A. Ostrander, and Janet L. Stanford.
    - Manuscript under review by Cancer Epidemiology, Biomarkers and Prevention

Conclusion

Two of six tasks in the Statement of Work have been initiated and completed and Task 3 is underway, as projected in the original study proposal timeline. Tasks 3-6 are projected for completion in Year 2 of the training grant. Specifically, Task 1, genotyping of tagSNPs in selected inflammation-related genes has been successfully completed. For Task 2, genotype quality has been evaluated and data from multiple sources has been prepared and satisfactorily merged for analysis. For Task 3, the analysis of genetic polymorphisms in inflammation-related genes and their association with prostate cancer risk is currently underway.

Personnel Clarification

The original proposal listed Dr. Elaine Ostrander as Co-Investigator and Dr. Ziding Feng as a Collaborator in Year 2. These individuals were misclassified and, while they will provide the support and services originally described, their roles on this project are more appropriately defined as Consultants. They are committed to this research effort and Ms. Salinas' progress toward her career goals, but their contributions will be on an as needed basis and do not involve measurable effort.

References

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3. Scheet P, Stephens M. A fast and flexible statistical model for large-scale population genotype data: applications to inferring missing genotypes and haplotypic phase. Am J Hum Genet 2006;78:629-44.

4. Zhao LP, Li SS, Khalid N. A method for the assessment of disease associations with single-nucleotide polymorphism haplotypes and environmental variables in case-control studies. Am J Hum Genet 2003;72:1231-50.

Appendix I: Table of Genotyped Inflammation-related Genes and SNPs[1]

Gene	SNPs										
AKT1	rs1130214	rs2494738	rs2498804	rs9989156							
COX1	rs1326913	rs3842787	rs5789								
COX2	rs1119231	rs12042763	rs12401885	rs20415	rs20417	rs2066826	rs2206593	rs2745557	rs3918304	rs4648261	
	rs4648276	rs5270	rs5275	rs6425043	rs6685280	rs689462	rs689466	rs689470	rs964570		
CXCL12	rs1029153	rs1144483	rs1147882	rs11595460	rs1436927	rs1801157	rs197452	rs2002194	rs2236534	rs2297630	
	rs2505734	rs266076	rs2781551	rs2839685	rs2839689	rs2861442	rs3780891	rs6593412	rs77839	rs7904065	
CXCR4	rs10928558	rs11674937	rs11897084	rs12691874	rs13008147	rs13022389	rs16832995	rs16833158	rs17466699	rs2680880	
	rs2734871	rs4954574	rs6726457	rs6751768	rs9973445						
IL6	rs2069840	rs2069845	rs2069860								
IL6R	rs1386821	rs4075015	rs4341355	rs4845374	rs4845617	rs4845623	rs6427627	rs6667434	rs7514452	rs7518199	rs8192284
IL6ST	rs10940495	rs11574783	rs2228043	rs6870870							
IL6ST	rs11574783	rs2228043	rs6870870								
IL8	rs2227306	rs4073									
NFKB1	rs1020759										
PIK3R1	rs12652661	rs1445760	rs16897511	rs16897558	rs173702	rs1823023	rs2112208	rs2161120	rs2431166	rs251399	
	rs251406	rs251408	rs34303	rs34306	rs34309	rs3756668	rs3815701	rs4122269	rs6876003	rs706713	
	rs706716	rs7713645	rs831122	rs831125							
STAT3	rs1026916	rs1053005	rs2293152	rs2306580	rs3816769	rs4103200	rs7211777	rs744166	rs957970		
TNF	rs1799964	rs1800610	rs1800629	rs1800750	rs2799724	rs2857713	rs3093559	rs3093662	rs3093664	rs3093665	rs3093672
TNFSF6	rs929087										
VEGF	rs11758547	rs1547651	rs3025010	rs3025030	rs3025035	rs699947	rs833052	rs833053	rs833057	rs833058	
	rs833060	rs833068	rs9394963								

1 This list does not include 3 SNPs that failed on the ABI SNPlex genotyping platform.

Appendix II 2008 Program in Prostate Cancer Research Seminar

2008 PROGRAM IN PROSTATE CANCER RESEARCH SEMINARS 5:00-6:00 P.M. (3rd Thursday)

PPCR SEMINARS	SPEAKER	TITLE	Res #45112 SEMINAR LOCATION, 5 – 6pm	Res #58092 Individual Meetings, 8-4:30
January 17, 2008	**Mark Rubin, MD,** Professor, Dept. of Pathological & Laboratory Medicine, Cornell Weill Medical College	**"Gene Fusion Prostate Cancer an Update"**	FHCRC Weintraub Building, **Pelton Auditorium**	FHCRC **Arnold Building,** 1st Floor, **Room M1-A403**
February 21, 2008	**Speaker TBA**	FHCRC Weintraub Building, **Pelton Auditorium**	FHCRC **Arnold Building,** 1st Floor, **Room M1-A403**	
March 20, 2008	**Michael Weber, PhD,** Director, UVa Cancer Center, University of Virginia Health System	FHCRC Weintraub Building, **Pelton Auditorium**	FHCRC **Arnold Building,** 1st Floor, **Room M1-A403**	
April 17, 2008	**Ralph W. deVere White, MD,** Professor & Chairman, Dept. of Urology; Director, UC Davis Cancer Center, University of California, Davis School of Medicine	FHCRC Weintraub Building, **Pelton Auditorium**	FHCRC **Arnold Building,** 1st Floor, **Room M1-A403**	
May 15, 2008	**Shoshana Yakar, PhD,** Mt. Sinai School of Medicine, NYC	FHCRC Weintraub Building, **Pelton Auditorium**	FHCRC **Arnold Building,** 1st Floor, **Room M1-A403**	
June 19, 2008	**Adam S. Kibel, MD,** Associate Professor, Div. of Urologic Surgery, Director of Urologic Oncology, Washington University School of Medicine	FHCRC Weintraub Building, **Pelton Auditorium**	FHCRC **Arnold Building,** 1st Floor, **Room M1-A403**	
July 17, 2008	**Philip Kantoff, MD,** Professor of Medicine, Medical & Solid Tumor Oncology, Harvard Medical School	FHCRC Weintraub Building, **Pelton Auditorium**	FHCRC **Arnold Building,** 1st Floor, **Room M1-A403**	
August 21, 2008	**Speaker TBA**	FHCRC Weintraub Building, **Pelton Auditorium**	FHCRC **Arnold Building,** 1st Floor, **Room M1-A403**	
Sept. 18, 2008	**Arul Chinnaiyan, MD, PhD,** Professor of Pathology & Urology; Director of Pathology Research Informatics & Cancer Bioinformatics, University of Michigan	FHCRC Weintraub Building, **Pelton Auditorium**	FHCRC **Arnold Building,** 1st Floor, **Room M1-A403**	
October 16, 2008	**Anthony V. D'Amico, MD, PhD,** Chief, Genitourinary Radiation Oncology, Brigham & Women's Hospital	FHCRC Weintraub Building, **Pelton Auditorium**	FHCRC **Arnold Building,** 1st Floor, **Room M1-**	

			A403
Nov. 20, 2008	**Peter Carroll, MD**, Associate Dean, UCSF School of Medicine; Chair, Dept. of Urology	FHCRC Weintraub Building, **Pelton Auditorium**	FHCRC **Arnold Building,** 1st Floor, **Room M1-A403**
Dec. 18, 2008	**Peter Scardino, MD,** Chair, Dept. of Surgery, Florence and Theodore Baumritter/Enid Ancell Chair of Urologic Oncology, Memorial Sloan-Kettering Cancer Center	FHCRC Weintraub Building, **Pelton Auditorium**	FHCRC **Arnold Building,** 1st Floor, **Room M1-A403**